



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of

VAN DEN BRINK et al

Atty. Ref.: 4560-4

Serial No. 10/518,414

TC/A.U.: 1636

Filed: August 30, 2005

Examiner: Joike, M.K.

For: IMPROVED METHOD OF PRODUCING AN ASPARTIC PROTEASE  
IN A RECOMBINANT HOST ORGANISM

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February 13, 2009

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Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**APPEAL BRIEF**

Sir:

Appellants hereby appeal the final rejection of claims 1-5, 8-15, 17-19, 22 and 23, in the Office Action dated May 13, 2008, and submit the present Appeal Brief pursuant to 37 CFR § 41.37. A Notice of Appeal was filed September 15, 2008, the date for filing an Appeal Brief having been extended up to February 15, 2009, by submission of the required petition and fee herewith.

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**(I) REAL PARTY IN INTEREST**

The real party in interest is Chr. Hansen A/S a corporation of the country of Denmark, by way of an Assignment from the inventors to Chr. Hansen A/S, Bøge Allé 10-12, Hørsholm, Denmark DK-2970, recorded in the U.S. Patent and Trademark Office on August 30, 2005, at Reel 017497, Frame 0355.

**(II) RELATED APPEALS AND INTERFERENCES**

Appellants, Appellants' legal representative, and the assignee are not aware of any related prior or pending appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

**(III) STATUS OF CLAIMS**

Claims 1-15 and 17-23 are pending. Claims 1-5, 8-15, 17-19, 22 and 23 have been finally rejected. Claims 6, 7, 20 and 21 stand objected to but are indicated as being allowable if rewritten in independent form. Claim 16 has been cancelled.

Claims 1-5, 8-15, 17-19, 22 and 23 are the subject of the present appeal. A copy of claims 1-5, 8-15, 17-19, 22 and 23 is attached as a Claims Appendix, pursuant to Rule 41.37(c)(1)(viii).

**(IV) STATUS OF AMENDMENTS**

An Amendment Under Rule 116 was filed December 12, 2008 in response to the final Office Action dated May 13, 2008. In the Advisory Action dated January 28, 2009, the Examiner indicated that the request for reconsideration presented in that Amendment had been considered.

**(V) SUMMARY OF CLAIMED SUBJECT MATTER**

The present invention, as claimed in claim 1 (and claims 2-5, 8-15, 22 and 23 which depend therefrom), relates to a process for producing an isolated polynucleotide sequence encoding a modified polypeptide. The process comprises: i) modifying a polynucleotide sequence that comprises a DNA sequence encoding a polypeptide comprising an aspartic protease amino acid sequence to encode an extra polypeptide N-X-T glycosylation site in the aspartic protease amino acid sequence; and ii) isolating the polynucleotide sequence resulting from step (i) which isolated polynucleotide sequence encodes the modified polypeptide. By incorporating an extra N-X-T glycosylation site into a polypeptide comprising an aspartic protease amino acid sequence, the recombinant production capacity of aspartic protease is increased.

Support for the above-described process is found, for example, at page 5, lines 29-34. Page 6, lines 1-3 indicate what is intended by “modifying” a polynucleotide sequence that comprises a DNA sequence encoding a polypeptide and page 6, lines 6-9 indicate what is intended by the term “extra” in step (i) above (see also page 10, lines 18-22). –N-X-T glycosylation site is defined at page 10, lines 10-16. Page 6, lines 11-30, indicate what is intended by “an aspartic protease amino acid sequence” (see step (i) above) and page 6, line 32 to page 7, line 4 indicate what is intended by the recitation in step (ii) of “isolated polynucleotide sequence.” Support for the above-described process is also found, for example, in claim 1 as originally filed.

In a further embodiment, the present invention, as claimed in claim 17 (and claims 18 and 19 which depend therefrom), relates to an isolated polypeptide exhibiting aspartic protease

activity. The polypeptide comprises a N-X-T glycosylation site. In accordance with this embodiment, the aspartic protease is a chymosin. Support for this aspect of the invention can be found, for example, at page 7, lines 29 and 30, what is intended by “an isolated polypeptide” being indicated at page 7, lines 32-34. Further support for this embodiment can be found, for example, in original claims 16 and 17.



**(VI) GROUND OF REJECTION TO BE REVIEWED ON APPEAL**

The following grounds of rejection are presented for review:

Whether claims 17-19 are anticipated under 35 USC 102(b) by USP 6,127,142.

Whether claims 1-5, 8, 12-15, 17-19 and 22 would have been obvious under 35 USC 103(a) over USP 5,800,849 in view of Kasturi et al (Biochem. J. 323:415-419 (1997)), and further in view of USP 6,127,142.

Whether claims 9-11 and 23 would have been obvious under 35 USC 103(a) over USP 5,800,849, Kasturi et al (Biochem. J. 323:415-419 (1997)), USP 6,127,142 and further in view of Korman et al (Curr. Genet. 17:203-212 (1990)).

**(VII) ARGUMENT**

**REJECTION OF CLAIMS 17-19 UNDER 35 USC 102(b) AS ANTICIPATED BY  
USP 6,127,142**

The subject matter of claims 17-19 is not anticipated by USP 6,127,142.

Accordingly, reversal of the rejection is respectfully requested.

Claim 17, from which claims 18 and 19 depend, is drawn to an isolated polypeptide that exhibits aspartic protease activity and that comprises an N-X-T glycosylation site. The claim specifically requires that the aspartic protease be a chymosin.

USP 6,127,142 relates to a method for deglycosylating an aspartic protease from *Rhizomucor miehei* (EC 3.4.23.23 Mucor rennin), which is not a chymosin (EC 3.4.23.4). Indeed, the Examiner acknowledges this to be the case on page 3 of the Office Action dated May 13, 2008, lines 1 and 2. Accordingly, the novelty of claims 17-19 over the reference is clear.

In maintaining the rejection, the Examiner refers to the statement at column 6, lines 38-41 of USP 6,127,142 which reads as follows:

Thus, as an example, a suitable milk clotting enzyme should ideally have an activity ratio similar to or close to that of pure calf chymosin for milk clotting activity at two different pH values such as 6.0/6.5 or 6.5-7.0.

The Examiner contends on page 3 of the May 13, 2008 Office Action that this sentence implies that bovine chymosin is acceptable to use as the protease. This assertion ignores the fact that USP 6,127,142 relates to a method of deglycosylating a protease that is not a

chymosin. The statement quoted above does not alter that fact – the quoted passage merely indicates a target activity ratio for the deglycosylated *Rhizomucor miehei* aspartic protease. This is clear from the sentence that follows at column 6, lines 43-48 of USP 6,127,142 which reads as follows:

Thus, it was found during the experimentation leading to the present invention that treatment of certain *Rhizomucor miehei* aspartic proteases having a relatively high pH dependency (ie. activity ratios above that of chymosin) with Endo H reduced the pH 6.0/6.5 or the 6.5/7.0 activity ratios to values closer to that of calf chymosin.

(It should be noted that “activity ratio” is not the same as “activity”).)

It will be clear from the foregoing that USP 6,127,142 does not anticipate the subject matter claimed in claims 17-19.

USP 6,127,142 would also not have rendered the invention claimed in claims 17-19 obvious. USP 6,127,142 teaches that homologous *Rhizomucor miehei* aspartic protease acquires significantly enhanced milk clotting activity when it is deglycosylated, likewise, heterologous *Rhizomucor miehei* aspartic protease produced in *A. oryzae*. The aspartic protease from *Rhizomucor miehei* is not a chymosin. Thus, nothing in the patent teachings could have suggested the claimed polypeptide exhibiting aspartic protease activity and comprising a N-X-T glycosylation site since the claims require that the aspartic protease be a chymosin.

In view of the above, reversal of the rejection is requested.

REJECTION OF CLAIMS 1-5, 8, 12-15, 17-19 AND 22 UNDER 35 USC 103(a) AS  
OBVIOUS OVER USP 5,800,849 IN VIEW OF KASTURI ET AL AND USP  
6,127,142.

The subject matter of claims 1-5, 8, 12-15, 17-19 and 22 would not have been obvious over the combination of USP 5,800,849, Kasturi et al and USP 6,127,142. Thus, reversal of the rejection is requested.

Underlying the present invention was a desire to provide a method for more efficiently producing aspartic protease in a host organism. The cited combination of art would in no way would have suggested addressing that problem by modifying the polynucleotide sequence to encode an (extra) N-X-T glycosylation site in the aspartic protease amino acid sequence, and subsequently expressing the sequence. The comments on page 5 of the May 13, 2008 Office Action, lines 10-15, suggest that the Examiner did not disagree with Appellants in this regard but that she was of the view that some other advantage "would flow naturally from following the suggestion of the prior art." In the Amendment filed December 12, 2008, Appellants urged the Examiner to provide a clear explanation as to the nature of the "other" advantage that the Examiner believed "would naturally flow from following the suggestion of the prior art." In the Advisory Action dated January 28, 2009, the Examiner responded as follows:

The point of the Examiner's statement was that the Examiner did not have to have the same motivation or "advantage" as the Applicants. The Examiner had another motivation or "advantage", as taught by the references, for combining them. The motivation is that Kasturi et al teach that N-linked glycosylation usually occurs at N-X-S/T sites, and N-glycosylation profoundly affects a protein's expression and function.

The flaw in this argument will be clear from the comments that follow.

USP 5,800,849 relates to a process for producing cheese in improved yield. The process comprises adding to milk a recombinant aspartic protease derived from *Rhizomucor miehei* to effect clotting. The reference indicates at, column 2, lines 57-60, that increased glycosylation was found to be surprisingly advantageous. Specifically, it is stated that:

The extent of glycosylation of the recombinant aspartic protease has surprisingly been found to be higher than the glycosylation of the aspartic proteases obtained from naturally occurring *Rhizomucor* strains.

As the Examiner has acknowledged (see page 5, last line of Office Action dated September 18, 2007), the reference does not teach the glycosylation site as being N-X-T. The higher degree of glycosylation was obtained by expression of the *Rhizomucor* gene in *Aspergillus*/*Trichoderma* host cells.

Kasturi et al reports the results of studies designed to compare “the impact of the Xaa residue in Asn-Xaa-Ser sequons with that of Asn-Xaa-Thr sequons to define further the protein sequences that control N-linked core glycosylation” (page 415, right column, bottom of only full paragraph). Rabies virus glycoprotein (RGP) was the primary model system used.

USP 6,127,142 relates to at least partially deglycosylated microbially-derived milk clotting enzymes, particularly to aspartic proteases derived from *Rhizomucor miehei* having improved milk clotting activity. It is indicated at column 2, line 46, that decreased glycosylation was surprisingly advantageous. Specifically, it is stated that:

It has now surprisingly been found that homologous *Rhizomucor miehei* aspartic protease, contrary to what has been stated in the prior art, acquires a significantly enhanced milk clotting activity when it is deglycosylated, and furthermore, that the milk clotting activity of heterologous *Rhizomucor miehei* aspartic protease as produced in *Aspergillus oryzae* ... is enhanced significantly by deglycosylation.

On page 6 of the September 18, 2007 Action, the Examiner makes reference to column 3, lines 38-41 of USP 6,127,142 and contends that this passage implies “that bovine chymosin is acceptable to use as the protease”. Appellants explain above, in connection with the rejection of claims 17-19, why this comment reflects a misunderstanding on the Examiner’s part of the reference teachings .

Summarizing, USP 5,800,849 teaches that “glycosylation of the aspartic protease can give an increase in cheese yield” (column 1, lines 25-27 – underlining added), Kasturi et al provides a comparison of “the impact of the Xaa residue in Asn-Xaa-Ser sequons with that of Asn-Xaa-Thr sequons to define further protein sequences that control N-linked core glycosylation (p. 415, right column, bottom only full paragraph) and USP 6,127,142 teaches that homologous *Rhizomucor miehei* aspartic protease acquires significantly enhanced milk clotting activity when it is deglycosylated, likewise heterologous *Rhizomucor miehei* aspartic protease produced in *Aspergillus oryzae*.

As pointed out above, the Examiner’s comments at page 5 of the May 13, 2008 Action (lines 10-15) suggest that some advantage “would flow naturally from following the suggestion of the prior art.” The Examiner’s explanation as to the nature of that advantage is set out in the Advisory Action, as quoted above. Respectfully, the

Examiner's explanation as to why one skilled in the art would have desired to use a N-X-T-glycosylation site in chymosin is not consistent with the teachings of the cited art, including the teaching of USP 6,127,142 of significantly enhanced clotting activity associated with deglycosylated aspartic protease. It was Appellants, not the art, who taught the advantage of such use.

Appellants submit that the rejection is clearly based on improper hindsight-based reasoning. Nothing in the citations would have suggested their combination and nothing in the combination (even if made) would have suggested the present invention.

Accordingly, reversal of the rejection is requested.

REJECTION OF CLAIMS 9-11 AND 23 UNDER 35 USC 103(a) AS OBVIOUS OVER  
USP 5,800,849, KASTURI ET AL, USP 6,127,142 AND KORMAN ET AL

The subject matter of claims 9-11 and 23 would not have been obvious over the combination of USP 5,800,849, Kasturi et al, USP 6,127,142 and Korman et al. Thus, reversal of the rejection is requested.

Claims 9-11 are dependent claims directed the production of the protease as a fusion protein, and 23 is directed to the use of Aspergillus host organisms.

In the Amendment filed February 19, 2008, Appellants indicated that the Examiner's position was not understood. Appellants do not find clarification in the Advisory Action.

Appellants submit that nothing in the references would have provided the motivation necessary to arrive at the present invention. The deficiencies of USP 5,800,849, Kasturi et al and USP 6,127,142 are discussed above. Korman et al adds

nothing that would have cured those failings or brought one skilled in the art closer to the present invention. It is only with the benefit of the present invention that the citations would have been combined and their combination, even if made, would not have suggested the subject matter of the instant claims. Accordingly, reversal of the rejection is requested.

**CONCLUSION**

In conclusion it is believed that the application is in clear condition for allowance; therefore, early reversal of the Final Rejection and passage of the subject application to issue are earnestly solicited.

Respectfully submitted,

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(VIII) CLAIMS APPENDIX

1. A process for producing an isolated polynucleotide sequence encoding a modified polypeptide comprising: i) modifying a polynucleotide sequence that comprises a DNA sequence encoding a polypeptide comprising an aspartic protease amino acid sequence to encode an extra polypeptide N-X-T glycosylation site in the aspartic protease amino acid sequence; and ii) isolating the polynucleotide sequence resulting from step (i) which isolated polynucleotide sequence encodes the modified polypeptide.

2. The process for producing an isolated polynucleotide sequence of claim 1, wherein the aspartic protease is a chymosin.

3. The process for producing an isolated polynucleotide sequence of claim 2, wherein the chymosin is a mammalian chymosin.

4. The process for producing an isolated polynucleotide sequence of claim 3, wherein the mammalian chymosin is bovine chymosin.

5. The process for producing an isolated polynucleotide sequence of claim 2, wherein the polypeptide comprising an aspartic protease amino acid sequence is selected from the group consisting of pre-prochymosin, prochymosin and mature chymosin.

8. The process for producing an isolated polynucleotide sequence of claim 1, wherein the modified polypeptide comprises, within the aspartic protease amino acid sequence, an artificial linker comprising a N-glycosylation site.

9. The process for producing an isolated polynucleotide sequence of claim 1, wherein the polypeptide comprising an aspartic protease amino acid sequence comprises a fusion protein wherein the aspartic protease amino acid sequence is connected to a fusion partner.

10. The process for producing an isolated polynucleotide sequence of claim 9, wherein the fusion partner is selected from the group consisting of glucoamylase, alpha-amylase, cellobiohydrolase and a part thereof.

11. The process for producing an isolated polynucleotide sequence of claim 8, wherein the polypeptide comprising an aspartic protease amino acid sequence comprises a fusion protein that comprises the aspartic protease amino acid sequence connected to a fusion partner, which fusion partner is selected from the group consisting of glucoamylase, alpha amylase, cellobiohydrolase and a part thereof, and wherein the artificial linker is situated between a pro-sequence and the fusion partner.

12. An isolated polynucleotide sequence encoding a modified polypeptide obtainable by the process of claim 1.

13. A method of producing a modified polypeptide exhibiting aspartic protease activity comprising the steps of cultivating a host organism comprising the isolated

polynucleotide sequence of claim 12 so that said modified polypeptide is produced and isolating the produced modified polypeptide exhibiting aspartic protease activity.

14. The method of producing a modified polypeptide of claim 13, wherein the host organism is a yeast cell or a filamentous fungal cell.

15. The method of producing a modified polypeptide of claim 14, wherein the host organism is a filamentous fungal cell and the filamentous fungal cell is an *Aspergillus* cell.

17. An isolated polypeptide exhibiting aspartic protease activity comprising a N-X-T glycosylation site, wherein the aspartic protease is a chymosin.

18. The isolated polypeptide of claim 17, wherein the chymosin is a mammalian chymosin.

19. The isolated polypeptide of claim 18, wherein the mammalian chymosin is bovine chymosin.

22. The process for producing an isolated polynucleotide sequence of claim 8 wherein the N-glycosylation site is a N-X-T glycosylation site.

23. The method of producing a modified polypeptide of claim 15, wherein the *Aspergillus* cell is an *Aspergillus niger* cell or an *Aspergillus niger* var. *awamori* cell.

**(IX) EVIDENCE APPENDIX**

(NONE)

(X) **RELATED PROCEEDINGS APPENDIX**

(NONE)